Ultrastructural observation on the response of equine hoof defects to dietary supplementation with Farrier's Formula

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Farrier's Formula feed supplement was added to the diet of 18 horses with two types of hoof horn defects. The first group of horses showed sand cracks and crumbling horn around the nail holes; the second group suffered frequent bruising and had flat feet with collapsed heels. Hoof clippings from both groups were studied in the transmission and scanning electron microscopes. All the horses showed a progressive improvement in the gross and microscopic structure of the hoof horn, starting six weeks after the supplementation began. Once good quality hoof horn had grown there was no relapse during the two year period of the study.

THE primary purpose of the horse's hoof is to provide a protective capsule of horn surrounding the terminal phalanges. When the foot strikes the ground some form of shock absorption is necessary, and it is provided in part by the specialised contents of the hoof capsule, eg, the hoof cartilages and the digital cushion, and also by the structure of the horn itself. Defects in the hoof horn reduce its functional integrity and are a major cause of reduced performance and suffering to the horses concerned. The types of gross defect range from thin, friable hoof horn with large crumbling areas around the nail holes (Comben and others 1984, Kempson 1987) to a more clinically normal hoof capsule which none the less shows a persistent, widespread bruising. The first type of defect is easily identified directly, but the second type is revealed by a shortening of the stride, intermittent lameness, tenderness on hard or stony ground and often an unwillingness to jump. Horses with the second type of defect do not always lose their shoes as often as horses with the first type of defect, which can lead to an incorrect diagnosis of navicular disease (J. M. Killingbeck, personal communication) or arthritis (L. M. Brown, personal communication).

Nutrition is now being recognised as an important factor in the growth of healthy hoof with a normal structure. Studies by Comben and others (1984) showed that dietary supplementation with the B-group vitamin biotin resulted in varying degrees of improvement in the hoof horn. Further studies (Kempson 1987) found a second group of horses, including many which had failed to respond to biotin supplementation, which showed improvements after the addition of calcium and increased protein to the diet.

The purpose of this study was to monitor the effect of Farrier's Formula (Life Data Labs, Cherokee, Alabama) on the growth and structure of the hoof horn. Farrier's Formula had been specifically developed as a feed supplement for horses with hoof horn defects. Hoof horn clippings removed during routine attention by the farrier were examined in the scanning and transmission electron microscopes.

Materials and methods

Eighteen horses were used in the study (Table 1). All of them had been referred with a history of gross horn defects or problems of poor horn growth and lameness which had persisted for between seven months (horse 6) and two-and-a-half years (horse 12) since the owner had noticed the changes. Farrier's Formula was supplied to the owners of the horses and added to the normal concentrate feed at the rate of 170 g/day; the nutrients supplied by each dose are listed in Table 2. The owners were asked to omit bran from the diet. The majority of the horses found the Farrier's Formula highly palatable and some horses out at grass would eat the supplement on its own. Only horse 2 found it unpalatable but was persuaded to eat it mixed with molasses. Horses 1 to 7 have been monitored for two years and the others for more than one year. Hoof clippings removed during routine attention by the farrier were examined in the scanning and transmission electron microscopes.

TABLE 1: Breed, age, sex and hoof defects of the horses studied

Horse	Breed/type	Age	Sex	Hoof defects
1	Thoroughbred	5	G	When shod as a four-year-old, went lame, tender and short striding on hard or stony ground. Very poor growth. Examination showed no pathology within the hoof capsule. Intermittently lame for 18 months
2	Quarterhorse	8	G	Navicular disease diagnosed, large flat feet, collapsed heels, frequent shoe loss
3	Hunter	9	M	Sandcracks, crumbling around nail holes, frequent shoe loss
4	Thoroughbred	13	M	Intermittent lameness, short striding on hard or stony ground, widespread bruising in white line
5	Thoroughbred	6	M	Shoe loss every two to three weeks, feet became larger and flatter
6	Hanoverian	5	G	Sandcracks and crumbling horn around nail holes
7	Hanoverian X thoroughbred	15	G	Thin friable hoof horn, crumbling around nail holes, intermittent lameness
8	Connemara	3	G	Flaking horn, poor growth, bouts of lameness
9	Connemara	7	G	Crumbling horn, tender on hard ground, shoe loss every four to five days
10	Thoroughbred	7	M	Tender on stony ground, poor growth, shoe loss approximately every two weeks
11	Thoroughbred	15	G	Feet had become very flat and large, reluctant to move on hard ground, lame for several months
12	Hunter	17	G	Sandcracks and crumbling around nail holes
13	Anglo-arab	7	G	Sandcracks in all four feet, difficulty in retaining shoes
14	Hanoverian	6	M	Loss of shoes on average every seven days, bruising and corns, flat feet
15	Riding cob	9	G	Brittle horn around nail holes
16	Riding horse	8	G	Navicular disease, poor horn growth, collapsed heels
17	Native pony	15	G	Crumbling around nail holes, frequent loss of shoes
18	Hanoverian cross	3	M	Sandcracks, with poor growth

G Gelding
M Mare

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Electron microscopy

The observations were made on trimmings from the front feet. The cut surface opposite to the surface in contact with the shoe or ground was examined. A sample 1 to 2 cm long was cut from the toe region and processed for scanning electron microscopy, and a slice no more than 1 mm deep was cut immediately adjacent to this sample and processed for transmission electron microscopy.

Scanning electron microscopy. — The samples were placed in 3 per cent gluteraldehyde in 0·1 M sodium cacodylate buffer (pH 7·3) for 12 hours. The blocks were post-fixed in 1 per cent osmium tetroxide in distilled water for one hour, dehydrated in acetone and critical point dried; they were mounted on aluminium stubbs, coated with gold and palladium and viewed in a Philips SEM 50S.

Transmission electron microscopy. — The tissue slices were fixed in 3 per cent gluteraldehyde in 0·1 M sodium cacodylate buffer (pH 7·3) for three hours. The specimens were post-fixed in 1 per cent osmium tetroxide for one hour, dehydrated in acetone and embedded in araldite. Ultrathin sections were cut from three areas across the hoof trimming: the white line area, the middle part of the wall and the outer part of the wall. The sections were stained with Reynold's lead citrate and 50 per cent uranyl acetate in ethanol, and viewed in a Philips EM400.

TABLE 2: Nutrients supplied by the daily dose of 170 g Farrier's Formula

Nutrient	Quantity supplied (mg)
DL-methionine	6188
Biotin	5·25
Ascorbic acid	1290
Choline	487
Inositol	262
Proline	1050
Hydroxyproline	750
L-tyrosine	618
Copper	94
Zinc	262
Iodine	0·0064

Results

Gross structure

Ten of the horses (numbers 3, 6, 7, 8, 9, 12, 13, 15, 17 and 18) in the study had obvious horn defects with horn crumbling and breaking away around the nail holes with or without sand cracks extending from the coronary band to the ground surface. The owners of these horses complained that the shoes would

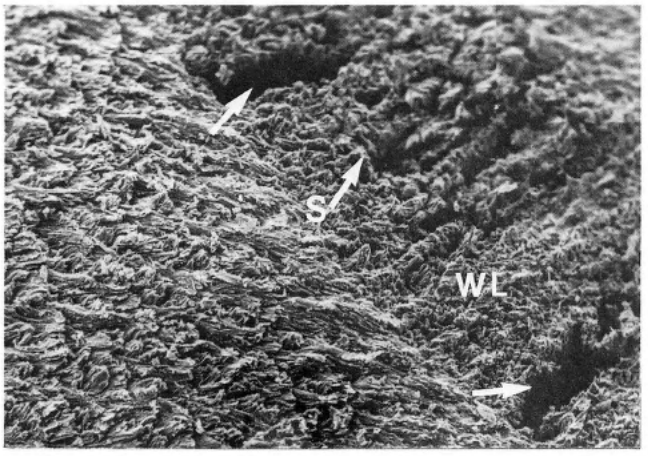

FIG 1: Scanning electron micrograph of the white line (WL) area shows numerous holes (↑) and loosely packed keratin squames (↑S). Horn adjacent to the white line contained no organised tubular structure. × 225

FIG 2: Normal structure was also absent from the stratum medium of the wall. Instead the keratin squames (↑) were loosely and randomly packed. × 1360

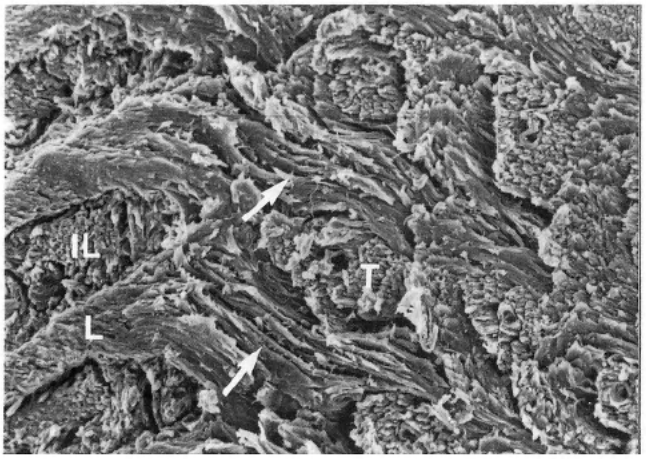

FIG 3: Three months after adding Farrier's Formula to the diet. The white line showed differentiation into lamina (L) and interlaminar horn (IL). The laminar horn (↑) could be seen spreading out among the horn tubules (T) of the stratum internum. × 225

FIG 4: Horn tubules (T) and intertubular horn (IT) in the stratum medium of the wall could be seen five to six months after Farrier's Formula had been added to the diet. The keratin squames formed a much closer association to one another. × 850

be retained for only four to seven days. The other eight horses did not show gross hoof horn problems but suffered frequent bruising, and tenderness or lameness on hard and stony ground; their feet had become flat, larger and with collapsed heels and some of them would lose their shoes every two to three weeks.

All the horses in the study showed improvement in the appearance and growth of the horn. The horses with visible defects such as sand cracks, showed the most rapid response to Farrier's Formula with new healthy horn visible growing down from the coronary band within six to 12 weeks. The shoes were retained for longer and the farriers commented on the increased strength of the wall. By six to nine months the sand cracks had grown out, the frequent loss of shoes had ceased, and the feet had a good shape and appearance. In the case of horse 17 the crumbling around the nail holes had virtually stopped by eight weeks.

The response of the second group of horses to Farrier's Formula was generally slower, although horses 4 and 11 showed a marked improvement by six weeks, becoming sound and working normally after several months of tenderness and lameness on roads, and retaining their shoes for longer. However, the first gross indication of improvement came approximately five months after starting on the formula when a growth ring appeared two-thirds of the way down the hoof wall. Distal to this growth ring the wall bulged outwards so that the distal hoof appeared to be bigger than the new horn. The bulge was present around the whole circumference of the foot and gave the quarters a 'winged' appearance. By seven to 12 months the bulge had grown out and the foot had a neater, more correct shape and sometimes a smaller shoe was required. The horses with collapsed horn at the heels improved steadily but horses 2 and 16, with navicular disease, required the longest period. Once good horn had grown the daily intake of Farrier's Formula was reduced to 85 g/day, and subsequently there was no relapse and the horses remained sound.

Ultrastructural observations

The scanning electron microscope observations taken before Farrier's Formula was introduced into the diet showed a loss of normal structure (Figs 1 and 2). There was no organised structure within the white line, holes were numerous and the keratin squames were loosely packed (Fig 1). The horn adjacent to the white line (Fig 1) had a homogeneous appearance of loosely packed keratin squames, and the normal structure of horn tubules surrounded by intertubular horn had been lost. Normal structure was also absent from the wall; there was no organisation, just loosely packed squames randomly arranged (Fig 2). Some of the samples did show horn tubules in the outermost layer of the wall.

The first signs of improvement appeared, six to eight weeks after first adding Farrier's Formula to the diet, in the white line region where differentiation into laminar and interlaminar

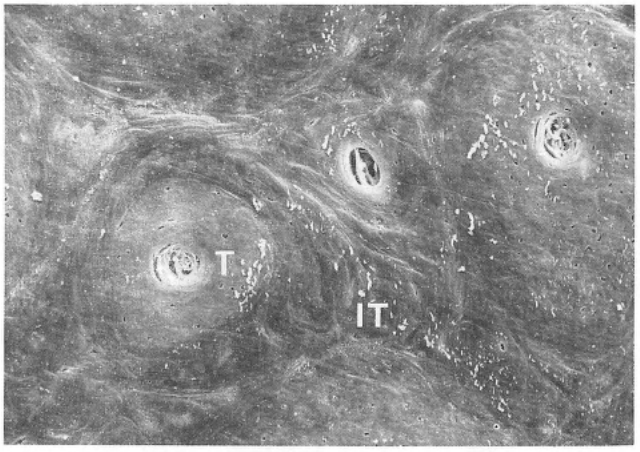

FIG 5: Micrograph from the same horse as Fig 1, one year after starting Farrier's Formula. The horn formed a dense, cohesive structure with well developed horn tubules and intertubular (IT) horn. × 850

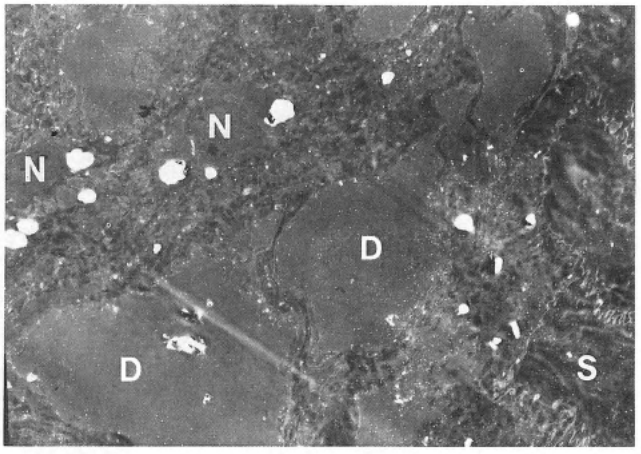

FIG 6: Transmission electron micrograph showing large areas of debris (D) and disruption in sections of the wall. Some of the squames showed parakeratosis with nuclei (N) present within the squames. Adjacent complete squames (S) contained poorly organised tonofilament bundles. × 1700

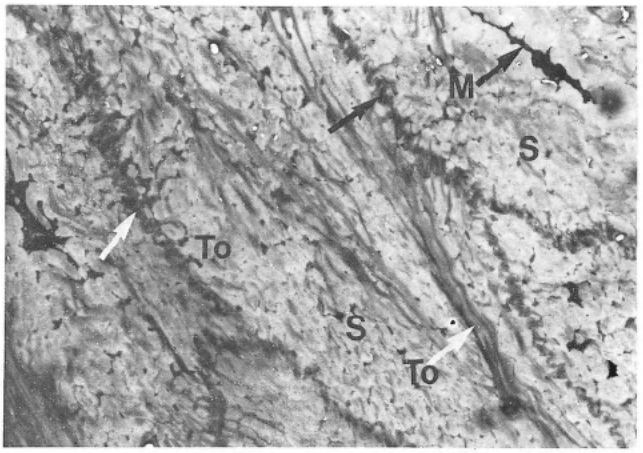

FIG 7: One year later than Fig 6 there was no evidence of disruption. The squames (S) were tightly packed and juxtaposed squame membranes (↑) showed an intimate association. Bundles of tonofilaments were orientated both parallel (↑To) and perpendicular (To) to the long axis of the squame. Electron dense matrix material (M) was present in the centre of the squames. × 3115

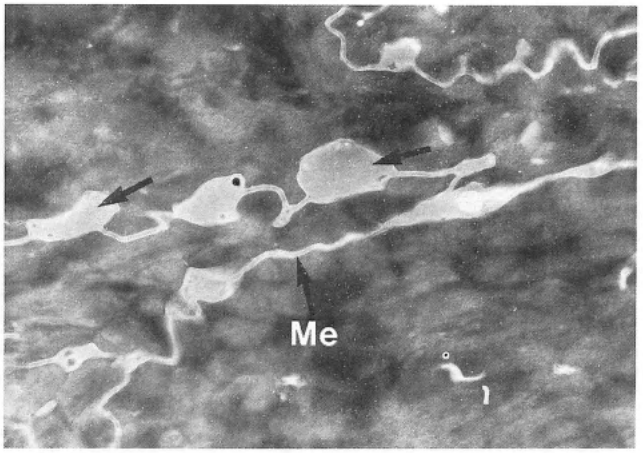

FIG 8: In some horses the damage was not as great as seen in Fig 6. The evidence of disruption in keratinisation was the presence of amorphous or membranous (↑) material in the inter-squame spaces. The material caused periodic separation of adjacent squame membranes (↑Me). × 10,200

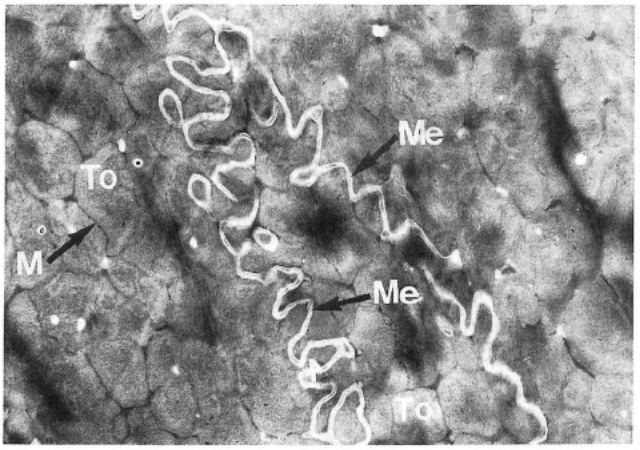

FIG 9: Nine months later an identical area from the same horse showed no intersquame material. The adjacent squame membranes (↑Me) were closely associated, forming a firm attachment to one another. Tonofilament bundles (To) were well organised and interspersed with electron dense matrix material (↑M). × 10,200

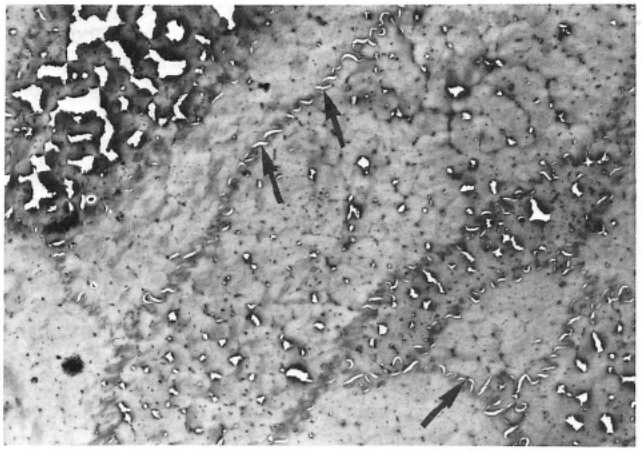

FIG 10: The third defect seen in the transmission electron microscope showed a breakdown of squame to squame attachment (↑) and a loss of internal organisation. By nine months hoof samples from these horses showed the same structure as in Fig 7. × 3115

horn could be observed. By three to four months the laminar horn showed a tighter more cohesive structure and was seen spreading out among the tubules in the adjacent wall area (Fig 3). The interlaminar horn, generated by the epidermis covering the distal tips of the sensitive laminae, showed some organised structure (Fig 3). By five to six months improvements were seen in the structure of the wall; the keratin squames were more closely associated, and horn tubules and intertubular horn could be seen (Fig 4). One year after the addition of the formula to the diet the structure of the horn was good and it formed a dense, cohesive structure with well developed horn tubules and intertubular horn (Fig 5). Individual keratin squames could not be identified.

The transmission electron microscope observations were made of the wall, and some of the samples showed areas of great disruption. In a section of hoof from horse 1 there were large areas of amorphous debris among the keratin squames some of which showed incomplete keratinisation with nuclei still present; the tonofilament bundles were poorly organised and sparse and the squames adjacent to the disrupted area also showed poor internal organisation (Fig 6). There was a progressive improvement in the structure of the hoof horn and 12 months later there was no evidence of debris and disruption; the squames were tightly packed and juxtaposed squame membranes showed an intimate association; tonofilament bundles packed the squames and were orientated both parallel and perpendicular to the long axis of the squame; electron dense matrix material was present in the centre of the squames and between bundles of tonofilaments (Fig 7).

Not all the horses showed such a degree of damage. More often there was just material present between adjacent squame membranes and sometimes this material had an amorphous appearance; on other occasions membranous configurations could be seen in the inter-squame material (Fig 8). Material was most commonly seen packed between adjacent squame membranes in the region of the wall adjacent to the white line. However, the intersquame material persisted longer in the outer third of the wall. Nine months after the sample shown in Fig 8 was taken, a similar area from the same horse showed no material in the region between adjacent squame membranes which were tightly pressed together and followed a tortuous course; bundles of tonofilaments were well organised compared with the earlier sample and matrix material which was more electron dense was interspersed between the bundles of tonofilaments (Fig 9).

The third type of defect seen in the transmission electron microscope showed a breakdown of the squame to squame attachments (Fig 10) and a loss of internal structure. By approximately nine months hoof samples from these horses showed the same structure as seen in Fig 7.

Discussion

Feeding Farrier's Formula to these horses resulted in an improvement in the gross structure and in the microscopic appearance of the hoof horn.

All the horses had a long history of hoof horn problems and some had been receiving feed supplements such as biotin for up to two years with no effect; they were kept in a wide variety of environments and used for different purposes. The study covered a long period during which the horses' intake of hard food would have varied according to the work the horse was doing and the time of year. The improvements seen after adding the formula to the diet were unaffected by the season of the year. In the author's experience healthy hoof horn is unaffected by prolonged drought, mud or the quality of bedding, whereas weak, poor quality hoof horn appears to lose its natural waterproofing properties and is more prone to environmental influences. The good quality hoof horn stimulated by the addition of Farrier's Formula was more able to withstand extremes of environment.

Previous studies (Kempson 1987) indicated that feeding bran can affect the calcium:phosphorus ratio of the diet. Four of the horses on the trial were fed bran before Farrier's Formula was fed and it is recognised that the improvement in the hoof horn structure of these horses may have been related to the change in the dietary calcium:phosphorus ratios. The owners of all the horses were desperate to find a solution to a long-term debilitating problem and were looking for the best possible solution. Unfortunately, it was impossible to match the trial horses, and their diets and activities with control horses, but the many hoof samples obtained from horses not receiving the formula give many data for comparison with the results of this study.

The presence of areas of debris and incomplete keratinisation in some of the horn samples implied that there had been episodic changes in epidermopoiesis. Intercellular oedema of the epidermis (Yager and Scott 1985) with widening of the intercellular spaces was found to be a feature of acute or subacute inflammation of the dermis, and was accompanied by disruption of the basement membrane zone (Yager and Scott 1985). The close physiological interdependence between epidermis and dermis (corium) results in epidermal change even when the epidermis is not the organ primarily affected. The material found in the intersquame spaces is thought to have been the result of inflammation in the underlying coria. Studies of young pigs (Kempson and Johnston 1990) with laminitis showed disruption of the basement membrane region and intercellular oedema within the epidermis. Observations on the adjacent horn (stratum corneum) of the pigs showed similar intersquame material.

The inflammation in the feet of the horses was thought to be the result of mechanical trauma, or bruising, or a subclinical laminitis. The inflammation had led to defects in the horn and a loss of functional integrity which rendered the foot more liable to further bruising and a continuous cycle of deterioration. Farrier's Formula apparently broke the cycle and stimulated the growth of good quality horn to protect the underlying soft tissues.

The presence of nuclei within the keratin squames indicated that the horn had become parakeratotic. Focal parakeratosis results in a vertical defect in the stratum corneum and can be found in any chronic dermatosis, and diffuse parakeratosis is consistent with zinc-responsive and vitamin A responsive dermatoses and ectoparasitism (Yager and Scott 1985). It is possible that the horses with parakeratosis responded to the zinc in the supplement even though there were no skin indications of zinc or vitamin deficiencies.

Improvements in the gross structure of the hoof horn were apparent as early as six weeks after starting to feed the farrier's formula supplement. The scanning electron microscope showed the earliest improvements in the white line and stratum internum.

The hoof wall is a heterogeneous structure derived from epidermis and coria in different regions (Habermehl 1981). Weakness in one of the components, for example, in acute laminitis, can result in a breakdown of the whole structure (Stashak 1987). This study has shown that the reverse is also true; an improvement in the laminar and white line horn can reduce the disintegration of the wall relatively quickly. It is thought that a strengthening of the stratum internum helped to reduce the insult and injury to the soft tissues of the foot and good quality keratinisation occurred, particularly in horses developing the distal 'bulge' in the wall. The more compact, cohesive horn stimulated by the addition of the formula to the diet, grew distally behind the loosely organised weaker horn.

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References

COMBEN, N., CLARK, R. J. & SUTHERLAND, D. J. B. (1984) *Veterinary Record* **115**, 642

HABERMEHL, K. H. (1981) The Anatomy of the Domestic Animals, Vol 3. Eds R. Nickel, A. Schummer and E. Seiferle. Berlin, Paul Parey. p546

KEMPSON, S. A. (1987) *Veterinary Record* **120**, 568

KEMPSON, S. A. & JOHNSTON, A. M. (1990) Proceedings of the First World Congress in Veterinary Dermatology, Dijon (In press)

STASHAK, T. S. (1987). Adam's Lameness in Horses. 4th edn. Philadelphia, Lea and Febiger. p486

YAGER, J. A. & SCOTT, D. W. (1985) Pathology of the Domestic Animals. 3rd edn. Vol 1. Eds K. V. F. Jubb and P. C. Kennedy, N. Palmer. New York, Academic Press. p481